Susceptibility of Immature Stages of *Homalodisca coagulata* (Hemiptera: Cicadellidae) to Selected Insecticides

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ABSTRACT Susceptibility of immatures of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), to 10 insecticides that included chlorpyrifos, dimethoate, endosulfan, bifenthrin, cyfluthrin, esfenvalerate, fenpropathrin, acetamiprid, imidacloprid, and thiamethoxam was evaluated in the laboratory. All five instars were exposed to different doses of each foliar insecticide by the petri dish technique, whereas a systemic uptake method was used to assess the toxicity to imidacloprid and thiamethoxam. All test insecticides exhibited high toxicity to all immature stages of *H. coagulata* at concentrations below the field recommended rates of each insecticide. Although all five instars were susceptible to test insecticides, mortality was significantly higher in first instars than in the older immatures based on low LC_{50} values (ranging from 0.017 to 5.75 ng(AI)/ml) with susceptibility decreasing with each successive stage. Fifth instars were generally the least sensitive (LC_{50} values ranging from 0.325 to 216.63 ng(AI)/ml). These results show that mortality was directly related to age of the insect and suggest that chemical treatment at early stages is more effective than at late stages. Acetamiprid (neonicotinoid) and bifenthrin (pyrethroid) were the most toxic to all five instars, inducing most mortality within 24 h and showing lower LC_{50} values ranging from 0.017 to 0.686 ng/ml compared with other insecticides (LC_{50} values ranging from 0.191 to 216.63 ng(AI)/ml). Our data suggest that a diverse group of very effective insecticides are available to growers for controlling all stages of *H. coagulata*. Knowledge on toxicity of select insecticides to *H. coagulata* immatures may contribute to our understanding of resistance management in future for this pest by targeting specific life stages instead of the adult stage alone.

KEY WORDS glassy-winged sharpshooter, organophosphates, pyrethroids, neonicotinoids, resistance management

Over the past decade, the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has become an economically important pest of grapes and citrus in southern California. Adults remain a considerable threat in grape vineyards because they are the principal vectors of *Xylella fastidiosa*, a plant pathogenic bacterium that induces Pierce's disease (PD) in grapes (Purcell and Feil 2001), and other plant diseases, including almond leaf scorch, oleander leaf scorch, and phony peach disease (Redak et al. 2004). The effective management of *H. coagulata* adults and its associated plant diseases include a variety of control options, such as cultural, biological, physical, and chemical. Of these, insecticides have become an integral component in management strategies and regional contact programs to suppress populations of *H. coagulata* and to keep losses below economic injury levels in citrus and grapes.

Both immature and adult stages of *H. coagulata* occur together on grapes and citrus; therefore, it is important to determine the effectiveness of each insecticide against the five developmental stages of *H. coagulata*. Immature stages of glassy-winged sharpshooter may not constitute as great a risk as adults in transmitting *X. fastidiosa* because they are incapable of long-distance flight. However, they nonetheless contribute to potential feeding damage and represent the portion of the total *H. coagulata* population that will soon develop to the more mobile adult stage. Therefore, strategies for controlling *H. coagulata* should include evaluation of the efficacy of various insecticides against nymphal instars as well adult stages. The inclusion of insecticides that are more effective against immature stages such as insect growth regulators (IGRs) could provide an important tool for mitigating or delaying insecticide resistance. Knowledge on insecticidal effects against all life stages of an insect is valuable information for determining the timing of chemical control measures.

Previous studies of *H. coagulata* control have dealt with insecticidal effects on adult populations from three southern California regions (Prabhaker et al. 2006). In this report, dosage–mortality relationships of 10 insecticides for first through fifth instars of *H. coagulata* were assessed to better understand the full

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impact of insecticide treatments on the whole *H. coagulata* population. Knowledge of the relative sensitivities of various developmental life stages of a pest to insecticides can be useful for determining the most suitable time to apply insecticides, especially when coupled with phenology and demography data for *H. coagulata* (Castle et al. 2005). Control concentrated on the most susceptible life stage can reduce input costs, while keeping pest populations low before they increase in numbers. In general, egg and pupal stages are often more difficult to control, possibly because of their greater quiescence relative to nymphs or larvae. Larval/nymphal stages are usually more susceptible to insecticides. However, even larvae vary in their susceptibility to insecticides, earlier ages being more susceptible than older larvae (Prabhaker and Toscano 2006). Implementing control measures against the most susceptible stage may be economically viable to control in terms of using lesser amounts of insecticide applied. Because immature stages represent a large portion of the total *H. coagulata* population, the effect of insecticide applications on survivorship of all stages should be determined.

The impact of insecticides on the immature stages of *H. coagulata* has not been investigated to date. Knowledge on the activity of insecticides toward all stages of *H. coagulata* is important. The objective of this study was to evaluate the relative susceptibility of five instars of *H. coagulata* from Riverside, CA, to seven conventional and three neonicotinoid insecticides. Insecticides were chosen based on a previous study (Prabhaker et al. 2006) that showed effectiveness against adults to compare the relative potency of the same insecticides against all immature stages of *H. coagulata.* This information is important to enable more effective use of insecticides in management programs for *H. coagulata* through improved understanding of their activity profiles.

Materials and Methods

Insects. Several field collections of immatures of *H. coagulata* from Riverside were made from both untreated and insecticide-treated citrus orchards for purposes of evaluating toxicity to insecticides. Treated citrus (*Citrus sinensis* L. variety Valencia and *Citrus limon* Burm. f.) included trees from orchards that were exposed to systemic insecticides at least 2 yr before the current study. Both untreated and treated citrus trees were present in the vicinity of field 5 at Agricultural Operations on the University of California, Riverside campus. Insects collected in this region from both previously treated and untreated citrus trees were combined for toxicological tests with the assumption that no differences would be expected in insect responses to various insecticides after a 2-yr posttreatment period. However, at the time of collections of *H. coagulata* for this study, no active treatments were made on the citrus trees in field 5. Collections were made at different times during summer to include the five instars as they became available. Therefore, first instars were tested first and fifth instars were tested last. Collections of insects were made on the day of each bioassay test by using sweep net and bucket sampling devices from citrus orchards (Castle et al. 2005a). Insects were transported to the laboratory for bioassays in plastic bags. Plastic bags with collections of *H. coagulata* were placed in transfer cages containing citrus seedlings to allow the insects to feed before transfer into petri dishes for exposure to insecticides. Insects that were hopping or actively feeding were selected for each bioassay to minimize control mortality.

Insecticides. The insecticides tested were selected because they are used to control insect pests on various agricultural crops, including citrus and grapes as well as to represent various classes of insecticide chemistries. Additionally, the selected insecticides were tested against adult *H. coagulata* so that the responses of all life stages could be compared. The following commercial formulations of 10 insecticides were evaluated: conventional insecticides were endosulfan (Thiodan, FMC Corp., Philadelphia, PA), chlorpyrifos (Lorsban, Dow Elanco, Indianapolis, IN), dimethoate (BASF, Florham Park, NJ), bifenthrin (Capture, FMC Corp., Philadelphia, PA), cyfluthrin (Baythroid, Bayer, Kansas City, MO), esfenvalerate (Asana, DuPont Agricultural Products, Wilmington, DE), and fenpropathrin (Danitol, Valent USA Corp., Walnut Creek, CA). Evaluation of three neonicotinoids also was included with acetamiprid (Assail, DuPont Agricultural Products), imidacloprid (Admire, Bayer), and thiamethoxam (Platinum, Syngenta, Oxnard, CA). All insecticides were diluted with water on the day of testing to make a series of concentrations. At least five concentrations of each insecticide were used to obtain mortality that ranged from 5 to 95%. First and second instars were exposed to five concentrations, whereas third, fourth, and fifth instars were exposed to six concentrations.

Susceptibility Tests. The response of each of the five instars of *H. coagulata* to various concentrations of eight contact insecticides (chlorpyrifos, dimethoate, endosulfan, bifenthrin, cyfluthrin, esfenvalerate, fenpropathrin, and acetamiprid) was determined by using a petri dish bioassay technique. The petri dish bioassay was adapted from previous studies to test glassy-winged sharpshooter adults (Prabhaker et al. 2006) with no modifications. Freshly cut citrus leaf discs sized to fit the base of a petri dish were immersed in each concentration of each insecticide for 30 s. Dipped leaf discs were allowed to dry for 1 h and were then placed on agar beds (1.5%) in each 60-mm petri dish. Concentrations for each insecticide were determined by preliminary tests to establish mortality ranging from 5 to 95% mortality. Water-treated leaf discs served as controls for each test. For exposure of each immature stage to the treatments, five insects per petri dish were briefly (5–7 s) anesthetized in plastic vials with carbon dioxide and then transferred to petri dishes by gently shaking them onto each treated leaf disc. Control mortality was always below 5% with this method of handling insects. At least five replications of each concentration were included per bioassay for

Table 1. Comparison of dose–mortality responses expressed as LC_{50} values after 24-h exposure of five immature stages of *H. coagulata* to three conventional insecticides: chlorpyrifos, dimethoate, and endosulfan

Insecticide class	Compound	Instar	*n*	Slope ± SE	LC_{50} (ng(AI)/ml)[a] (95% FL)[b]	χ^2 (df)	g (0.95)[c]
Organophosphate	Chlorpyrifos	First	448	1.4 ± 0.05	0.360 (0.124–0.437)a	11.06 (4)	0.02
		Second	452	1.8 ± 0.08	0.544 (0.281–0.702)a	6.9 (4)	0.10
		Third	461	1.6 ± 0.07	1.86 (0.842–3.11)b	14.4 (4)	0.12
		Fourth	446	1.2 ± 0.04	2.04 (1.08–3.79)b	12.4 (4)	0.09
		Fifth	458	1.9 ± 0.21	2.32 (1.42–4.21)b	5.6 (4)	0.11
	Dimethoate	First	453	2.0 ± 0.12	0.191 (0.165–0.256)a	6.5 (4)	0.25
		Second	476	1.2 ± 0.11	0.652 (0.344–0.748)b	7.4 (4)	0.32
		Third	447	1.1 ± 0.14	1.28 (0.947–3.93)c	6.7 (4)	0.27
		Fourth	452	1.2 ± 0.21	2.19 (1.24–5.48)c	7.23 (4)	0.22
		Fifth	461	1.6 ± 0.08	3.63 (2.03–7.21)c	8.8 (4)	0.19
Cyclodiene	Endosulfan	First	445	2.1 ± 0.19	0.910 (0.803–1.77)a	5.6 (3)	0.20
		Second	456	1.9 ± 0.11	1.54 (1.06–2.15)a	7.9 (4)	0.14
		Third	459	1.8 ± 0.09	1.81 (1.12–2.68)a	9.4 (4)	0.23
		Fourth	463	2.4 ± 0.21	9.18 (7.94–11.86)b	8.7 (4)	0.29
		Fifth	462	2.6 ± 0.16	22.02 (17.49–26.71)c	8.1 (4)	0.18

[a] LC_{50} in micrograms per milliliter for endosulfan.
[b] LC_{50} values followed by the same letter are not significantly different based on overlap of 95% FL for a particular pesticide, across the five stages.
[c] Index of significance for potency estimation, g, will be substantially smaller than 1.0 and seldom >0.4 for good sets of data (Finney 1971, p. 79).

each insecticide with each bioassay replicated three times. Mortality of immature *H. coagulata* was recorded at 24 h. Insects were maintained at 27 ± 2°C and a photoperiod of 14:10 (L:D) h. Any insect that failed to move when touched or was immobile on its back was considered dead.

Toxicity of imidacloprid and thiamethoxam was assessed using an uptake bioassay technique for systemic insecticides as described in Prabhaker et al. (2005, 2006). This technique allows the uptake of imidacloprid and thiamethoxam through petioles of detached citrus leaves. Excised citrus stems with two terminal leaves were placed in five to six serial dilutions of both systemics in aquapiks for 48 h to allow uptake of the compound. After the uptake period, test leaves were transferred to aquapiks containing water only. Previous studies have shown that imidacloprid or thiamethoxam taken up by the leaf within a 48-h period was sufficient to allow insects to respond to the compound after 24- and 48-h exposure based on 100% mortality observations recorded at 72- and 96-h postexposure (data not shown), indicating the presence of sufficient amounts of imidacloprid in test citrus leaves over time. Various stages of *H. coagulata* were transferred into small clip cages that were attached to each leaf to allow exposure to treated leaves. At least five insects were enclosed per clip cage. Exposure time of the insects to imidacloprid and thiamethoxam was for 24 h similar to the foliar insecticide exposure.

Statistical Analysis. Data from all experiments were subjected to probit analysis using POLO (Russell et al. 1977, LeOra Software 1987) to generate the LC_{50} values expressed as nanograms per milliliter for all compounds except endosulfan, which was measured as micrograms per milliliter, along with 95% fiducial limits (FL). Results were considered statistically significant when there were no overlaps of fiducial limits (95%) of LC_{50} for a particular insecticide.

Results

Organophosphates. The two organophosphates chlorpyrifos and dimethoate were toxic to all life stages of the *H. coagulate*, even after a short exposure period of 24 h (Table 1). Additionally, mortality to both insecticides increased after a 48-h exposure in four of the five instars variably (data not shown). When immature insects were treated with chlorpyrifos, toxicity was apparent at every life stage with significantly higher toxicity against the first and second instars (LC_{50} = 0.36 and 0.54 ng(AI)/ml, respectively) compared with the older stages. No significant differences in toxicity were observed between the first and second instars. According to LC_{50} values, third, fourth, and fifth instars were almost equally susceptible to chlorpyrifos (LC_{50} = 1.86, 2.04, and 2.22 ng(AI)/ml, respectively). However, mortality was significantly different between the younger insects (first and second instars) compared with the older instars with differences ranging from five- to six-fold for the first instars and from three- to four-fold between second and third and fourth and fifth instars, respectively.

The greatest insecticidal activity for dimethoate was against the first instars (LC_{50} = 0.19 ng(AI)/ml), comparable to that of chlorpyrifos responses (Table. 1). Dimethoate was also highly toxic to second instars, indicated by a low LC_{50} (0.65 ng(AI)/ml) and was significantly different from that of first instars. Decreasing LC_{50} values were observed with increasing age of the immatures, as seen by highest LC_{50} value of 3.63 ng(AI)/ml for the fifth instars. When third instars were treated with dimethoate, susceptibility was intermediate (LC_{50} 1.26 ng(AI)/ml at 24 h) and was significantly different from that of first, second, and fifth instars. Mortality increased after 48 h in older four instars (data not shown), suggesting that LC_{50} values decreased with longer exposure. In general, toxicity of

Table 2. Comparison of dose–mortality responses expressed as LC_{50} values after exposure of five immature stages of *H. coagulata* to four pyrethroids at 24 h

Compound	Instar	*n*	Slope ± SE	LC_{50} (ng(AI)/ml) (95% FL)[a]	χ^2 (df)	g (0.95)[b]
Bifenthrin	First	448	3.3 ± 0.06	0.042 (0.028–0.041)a	9.2 (4)	0.28
	Second	447	2.7 ± 0.17	0.068 (0.059–0.079)b	6.9 (4)	0.15
	Third	452	2.8 ± 0.02	0.092 (0.083–1.15)c	6.4 (4)	0.18
	Fourth	451	2.2 ± 0.12	0.158 (0.094–1.44)c	8.4 (4)	0.29
	Fifth	457	1.9 ± 0.06	0.325 (0.228–1.85)c	9.4 (4)	0.22
Cyfluthrin	First	453	1.8 ± 0.09	0.623 (0.514–1.71)a	5.6 (3)	0.22
	Second	466	1.7 ± 0.11	1.74 (1.33–1.97)a	7.9 (4)	0.36
	Third	452	1.5 ± 0.12	7.01 (3.82–7.68)b	6.2 (4)	0.10
	Fourth	448	1.3 ± 0.10	9.37 (5.39–9.99)b	7.4 (4)	0.21
	Fifth	456	1.9 ± 0.30	17.95 (12.24–21.60)c	9.5 (4)	0.23
Esfenvalerate	First	447	1.6 ± 0.24	0.02 (0.011–0.083)a	5.4 (3)	0.14
	Second	459	1.4 ± 0.14	0.24 (0.162–0.691)b	7.1 (4)	0.27
	Third	451	1.5 ± 0.19	1.33 (0.702–2.23)c	6.8 (4)	0.34
	Fourth	462	1.8 ± 0.18	1.66 (1.06–2.98)c	9.5 (4)	0.20
	Fifth	449	2.0 ± 0.12	3.18 (2.22–3.87)c	8.7 (4)	0.11
Fenpropathrin	First	455	1.7 ± 0.22	0.143 (0.088–0.624)a	5.9 (3)	0.14
	Second	452	1.9 ± 0.14	2.65 (1.75–4.53)b	8.6 (4)	0.24
	Third	464	1.5 ± 0.11	5.46 (3.85–7.89)b	8.9 (4)	0.16
	Fourth	460	1.9 ± 0.18	24.46 (15.68–22.08)c	10.2 (4)	0.26
	Fifth	451	2.0 ± 0.14	25.85 (18.61–26.57)c	9.2 (4)	0.15

[a] LC_{50} values followed by the same letter are not significantly different based on overlap of 95% FL for a particular pesticide, across the five stages.

[b] Index of significance for potency estimation, g, will be substantially smaller than 1.0 and seldom >0.4 for good sets of data (Finney 1971, p. 79).

the two organophosphates, chlorpyrifos and dimethoate, to *H. coagulata* immatures was similar.

Cyclodiene. Although endosulfan is not registered for use against *H. coagulata*, the susceptibility of endosulfan against the immature stages was evaluated to represent the cyclodiene class. The responses of each instar to endosulfan expressed as LC_{50} values (micrograms per milliliter) are presented in Table 1. Endosulfan was toxic to all stages but was more so to first, second, and third instars (LC_{50} values ranging from 0.91 to 1.81 μg(AI)/ml at 24 h). No significant differences in toxicity were observed between the three younger instars to endosulfan. The LC_{50} values for the fourth and fifth instars were significantly higher than those of the younger stages at 9.18 and 22.02 μg(AI)/ml, respectively, at 24 h based on nonoverlap of 95% fiducial limits. The fifth instars were significantly less sensitive than the fourth instars. Mortality increased slightly with longer exposure after 48 h in the second, third, fourth, and fifth instars (data not shown). Endosulfan was highly toxic to all stages of *H. coagulata*, but at higher concentrations compared with chlorpyrifos and dimethoate.

Pyrethroids. The lethal concentrations for median mortality (LC_{50}) obtained from probit mortality regression data for the pyrethroids bifenthrin, cyfluthrin, esfenvalerate, and fenpropathrin are shown in Table 2. Data show treatment of the five instars of *H. coagulata* with bifenthrin resulted in increasing LC_{50} values with increasing age of the immatures. Both first and second instars were found to be the most susceptible instars to bifenthrin with low LC_{50} values of 0.04 and 0.07 ng(AI)/ml, respectively, compared with the older stages. The difference in toxicity to bifenthrin between the first and second instars was significant but by only two-fold based on nonoverlapping of fiducial limits. The fifth instar exhibited the highest LC_{50} value at 0.32 ng(AI)/ml, with an eight- and five-fold difference in toxicity compared with the first and second instars, respectively. No significant differences in the LC_{50} values were observed between the three older stages, third, fourth, and fifth instars. Similar to treatments with chlorpyrifos, dimethoate, and endosulfan, mortality increased after 24 h for the four older stages (data not shown).

Cyfluthrin was very active against *H. coagulata* immatures but at higher concentrations than those of bifenthrin (Table 2). Although the LC_{50} values were higher for cyfluthrin than those observed for bifenthrin for all five instars, the general trend of the most susceptible stage being the first instar, and fifth instars being the least sensitive, was similar (Table 2). However, unlike the responses of *H. coagulata* immatures to bifenthrin, the difference in toxicity between the first (LC_{50} = 0.62 ng(AI)/ml) and fifth instars (LC_{50} = 17.95 ng(AI)/ml) was significantly higher by 29-fold. No significant difference was observed in responses of first and second instars (LC_{50} = 1.70 ng(AI)/ml) to cyfluthrin. The LC_{50} values were higher to cyfluthrin with increasing age but were not significantly different between third and fourth instars. The fifth instars were the least sensitive to cyfluthrin compared with the four younger instars. Mortality increased over time to cyfluthrin similar to bifenthrin, chlorpyrifos, dimethoate, and endosulfan (data not shown).

The LC_{50} values for esfenvalerate ranged from a low of 0.02 ng(AI)/ml to a high of 3.18 ng(AI)/ml for the five immature stages of glassy-winged sharpshooter. Based on LC_{50} values alone, decreasing susceptibilities on the order of first > second > third > fourth > fifth were observed (Table 2). These results reflect the

Table 3. Comparison of dose–mortality responses expressed as LC_{50} values after exposure of five immature stages of *H. coagulata* to three neonicotinoids at 24 h

Compound	Bioassay technique	Instar	*n*	Slope ± SE	LC_{50} (ng(AI)/ml) (95% FL)[a]	χ^2 (df)	g (0.95)[b]
Acetamiprid	Petri dish	First	448	1.8 ± 0.18	0.017 (0.003–0.073)a	6.9 (4)	0.19
		Second	447	1.5 ± 0.31	0.110 (0.084–0.354)b	7.4 (4)	0.35
		Third	468	1.9 ± 0.15	0.412 (0.366–0.630)c	8.2 (4)	0.22
		Fourth	444	1.8 ± 0.24	0.548 (0.452–1.17)c	6.7 (4)	0.30
		Fifth	457	1.9 ± 0.11	0.686 (0.507–3.08)c	10.4 (4)	0.26
Imidacloprid	Systemic uptake	First	453	2.0 ± 0.26	3.22 (2.16–5.85)a	6.2 (4)	0.17
		Second	447	2.2 ± 0.17	11.68 (7.62–20.28)b	9.4 (4)	0.15
		Third	451	2.3 ± 0.18	39.48 (26.24–64.64)c	8.6 (4)	0.14
		Fourth	462	1.9 ± 0.13	71.32 (45.26–99.25)c	7.6 (4)	0.17
		Fifth	456	2.0 ± 0.18	182.74 (117.31–215.30)d	10.1 (4)	0.12
Thiamethoxam	Systemic uptake	First	458	1.7 ± 0.12	5.75 (4.19–12.47)a	9.8 (4)	0.31
		Second	449	1.9 ± 0.13	96.52 (64.48–91.51)b	6.9 (4)	0.11
		Third	452	2.0 ± 0.09	122.26 (97.25–229.04)c	7.9 (4)	0.38
		Fourth	463	2.1 ± 0.13	137.59 (107.61–291.93)c	8.0 (4)	0.21
		Fifth	454	2.0 ± 0.10	216.63 (154.77–362.22)c	8.2 (4)	0.16

[a] LC_{50} values followed by the same letter are not significantly different based on overlap of 95% FL for a particular pesticide, across the five stages.

[b] Index of significance for potency estimation, g, will be substantially smaller than 1.0 and seldom >0.4 for good sets of data (Finney 1971, p. 79).

same trend as was observed for bifenthrin and cyfluthrin. However, a striking difference was apparent in toxicity between first and fifth instars, with esfenvalerate showing a significant difference of 159-fold, suggesting that the first instars were extremely susceptible to esfenvalerate. Bifenthrin was also similarly toxic to first instars (LC_{50} = 0.05 ng(AI)/ml) but was only eight-fold less toxic to fifth instars (LC_{50} = 0.32 ng(AI)/ml). No significant differences in mortality were observed between third, fourth, and fifth instars. Both esfenvalerate and bifenthrin were the most toxic to first instars compared with chlorpyrifos, dimethoate, endosulfan and cyfluthrin. Mortality increased after 48-h exposure to esfenvalerate, similar to the other test compounds (data not shown).

First instars of *H. coagulata* were again the most susceptible among the immatures when exposed to fenpropathrin, compared with the later instars (Table 2). The LC_{50} value for the first instars was very low, at 0.14 ng(AI)/ml, with a significant increase in LC_{50} value at 2.65 ng(AI)/ml (19-fold) for second instars. Susceptibility decreased during later stages, with LC_{50} values ranging from 5.46 to 25.85 ng(AI)/ml for third, fourth, and fifth instars. Both second and third instars were similarly susceptible to fenpropathrin, which was significantly less than the susceptibility of the first instars. No significant differences in responses of fourth and fifth instars were observed to fenpropathrin. Similar to the significant difference in toxicity between first and fifth instars to esfenvalerate, a notable difference of 181-fold was observed to fenpropathrin between first and fifth instars. Increases in mortality to fenpropathrin were observed in later instars after 48 h (data not shown).

Neonicotinoids. The dose–mortality relationship for all five immature stages of *H. coagulata* to the neonicotinoids acetamiprid, imidacloprid, and thiamethoxam are shown in Table 3. The results of tests with acetamiprid for various instars indicate that each instar, in general, was very sensitive to this foliar neonicotinoid. High mortality (LC_{50} = 0.02 ng(AI)/ml) was observed when first instars were exposed to acetamiprid, indicating that this stage was the most susceptible compared with the older instars (Table 3). A significant difference of six-fold less mortality was observed between the first and second instars. The LC_{50} values were significantly higher for the third, fourth, and fifth instars, with toxic values ranging from 24- to 40-fold compared with first instars. The highest LC_{50} value of 0.68 ng(AI)/ml for fifth instar was not significantly higher than those of third and fourth instars. Mortality increased after 48 h in each instar (data not shown). These results demonstrated that first and fifth instars were the most and the least sensitive, respectively, to acetamiprid.

The LC_{50} values for the systemic neonicotinoid imidacloprid were significantly higher compared with the foliar neonicotinoid acetamiprid. Mortality increased with each succeeding instar, from the first to fifth (Table 3). First instars were the most susceptible and significantly different in sensitivity to imidacloprid than the four older stages. Toxicity differences were 4-, 12-, 22-, and 57-fold between second, third, fourth, and fifth instars, respectively. Second and third instars were similar in their responses to imidacloprid but significantly different from fourth and fifth instars. In general, younger *H. coagulata* immatures were more susceptible to imidacloprid than were the older instars. The LC_{50} values for imidacloprid were higher compared with the pyrethroids when tested against five instars; however, the values observed were still very low compared with field rates. Very few survivors were observed after 24-h exposure to imidacloprid in clip cages (data not shown).

Results of tests conducted with thiamethoxam against the five immature stages are also presented in Table 3. In general, compared with acetamiprid or imidacloprid, the LC_{50} values were higher for thiamethoxam, indicating less sensitivity to this particular neonicotinoid. A clear trend similar to acetamiprid

and imidacloprid treatments was apparent across all instars, i.e., the first instars being the most sensitive compared with older instars. First instars were 38-fold more sensitive than fifth instars. Based on nonoverlapping of 95% FL, the LC_{50} values for first and second instars were significantly lower than for third, fourth, and fifth instars, suggesting that they were more sensitive than the older instars. Although sensitivity to thiamethoxam decreased with increasing age, based on the overlap of 95% fiducial limits, the LC_{50} values for third, fourth, and fifth instars did not differ significantly. Mortality of second, third, fourth, and fifth instars increased after 48 h to thiamethoxam (data not shown).

Discussion

Generally, laboratory bioassays assessing insecticide toxicity are indispensable for large-scale screening of insecticidal activity, and they also provide a fairly accurate measurement of effectiveness on a test insect. Therefore, the current study was an evaluation done under laboratory conditions in petri dishes, while acknowledging the possibility that the results could be different in a field situation where immatures are not stationary on treated plants. However, the general pattern of response across instars in the field may be the same as that observed under laboratory conditions.

Previous studies on the use of insecticides to control *H. coagulata* focused primarily on adult control (Akey et al. 2001, Bethke et al. 2001, Prabhaker et al. 2006). Our results suggest that insecticides that were very effective against adults are also suitable to control immature stages of glassy-winged sharpshooter. The concentrations required for controlling all five immature stages of glassy-winged sharpshooter with 10 insecticides at a short exposure period of 24 h were determined in this study. In general, high mortality was observed in all five developmental stages to all insecticides evaluated, suggesting a relatively high degree of susceptibility to the 10 insecticides evaluated. Although all test insecticides were highly toxic to all glassy-winged sharpshooter immature stages, differences in sensitivities were observed between insecticides and life stages at the concentrations tested. For example, first instars were the most susceptible, regardless of the insecticide applied as shown by the lowest LC_{50} values (Tables 1–3), whereas the older fourth and fifth instars were the least susceptible based on highest LC_{50} values recorded to all insecticides. These results suggest that mortality seems to be negatively correlated to increasing age and size of instars in this species. The order of sensitivity of immatures based on LC_{50} values to the 10 test insecticides was identical; specifically, first $>$ second $>$ third $>$ fourth $>$ fifth. Therefore, the critical time of insecticide treatments perhaps should be directed against the earliest instars to be most effective.

The results from our study showing significantly greater mortality in early instars are not unexpected, because first instars were the youngest and the smallest compared with third, fourth, and fifth instars. The effects of age on the susceptibility of larvae to insecticides, including *Bacillus thuringiensis* or IGRs, have been studied in several insect species, including *Plodia interpunctella* (Hübner), *Ephestia cautella* (Walker), *Spodoptera frugiperda* (J.E. Smith), *Bemisia tabaci* (Gennadius), *Plutella xylostella* (L.), *Choristoneura rosaceana* (Harris), and *H. coagulata* (McGaughey 1978; Hornby and Gardner 1987; Prabhaker et al. 1989; Liu et al. 1995a,b; Prabhaker and Toscano 2006). In these studies, it was demonstrated that the early instars were more susceptible than were the older instars. The consistency in susceptibility responses of five instars of *H. coagulata* to 10 insecticides seems to be in agreement with these studies. This trend could be related to size, because older instars are larger than the younger instars and perhaps need higher quantity of toxin to obtain the same level of mortality. This trend also could be related to physiological differences between the younger and older larvae such as the presence of defense mechanisms that allow breakdown of insecticides. Perhaps the development of defense mechanisms in the form of metabolic enzymes may differ among instars, becoming better established with higher titers in older instars as body weight increases.

This study has demonstrated the potential importance of knowing the differences in sensitivities of the various developmental stages of glassy-winged sharpshooter to insecticides for determination of the best time to spray insecticides. Selection of correct timing to derive maximum benefit from that product is possible by aiming appropriate treatments against the most abundant stage(s). Our results have shown that each product has a slightly different spectrum of activity related to either mode of action or residual activity; thus, optimal timing may vary. Populations of glassy-winged sharpshooter in southern California citrus have been shown to have a predictable phenology with a discrete new generation produced each spring (Castle et al. 2005b). Therefore, more precise targeting of various developmental stages should be carefully considered. Selection of suitable timing of applications also may be necessary to fully exploit the mode of action of insecticides, e.g., with IGRs that are effective only against immature stages. Appropriate timing of chemical application is essential for effective control of *H. coagulata* in the field.

All test insecticides demonstrated high levels of activity against all immature stages of *H. coagulata.* The activity of endosulfan was the lowest compared with the other insecticides, based on higher LC_{50} values, but the concentrations tested were still toxic compared with field rates. Such a response to endosulfan also was observed against *H. coagulata* adults (Prabhaker et al. 2006). Both organophosphates, chlorpyrifos and dimethoate, exhibited similar toxicity across all stages of *H. coagulata.* In contrast, clear differences in the effectiveness of pyrethroids against *H. coagulata* immatures were evident in this study. The higher potency of bifenthrin compared with fenpropathrin was apparent based on the lower LC_{50} values against

every instar. But the bigger difference was observed when toxicity against fifth instars was compared, i.e., the difference in LC_{50} between the two compounds was 615-fold lower for fenpropathrin, indicating that bifenthrin was significantly more potent. Large differences in potency of neonicotinoids to immatures of *H. coagulata* were observed. Acetamiprid was the most potent, exhibiting toxicity ratios of 189- and 338-fold relative to imidacloprid and thiamethoxam, respectively, against the first instars. Acetamiprid was also very toxic across all five instars, not just the first instar. The difference in potency could be related to direct contact of foliarly applied acetamiprid compared with availability of imidacloprid or thiamethoxam to insects only after feeding. Our results show that in spite of such large toxicity differences between *H. coagulata* immatures to neonicotinoids, this chemical class was very effective against all immature stages. Although the reason(s) for differences in toxicity is not clear for either pyrethroids or neonicotinoids, they may be related to changes in permeability of the integument that affected absorption of the contact insecticides or due to differing sites of action for each insecticide during each life stage.

Our previous study showed high toxicity of these 10 insecticides against *H. coagulata* adults in laboratory evaluations (Prabhaker et al. 2006). Comparing toxicity of each insecticide against immatures relative to adults, most insecticides were less toxic to adults. The effects of certain insecticides tested here on *H. coagulata* immatures were more pronounced in their activity than when applied against adults. However, the relative potency of insecticides against all glassy-winged sharpshooter life stages was similar in ranking. For example, among neonicotinoids, acetamiprid was the most toxic followed by imidacloprid and thiamethoxam. No difference in the order of toxicity for the four pyrethroids was observed between *H. coagulata* immatures and adults, which was in the following order: bifenthrin > esfenvalerate > cyfluthrin > fenpropathrin (Prabhaker et al. 2006). Similarly, between the organophosphates, chlorpyrifos was slightly more toxic than dimethoate to all stages. Both previous and present results indicate that the relative toxicities of the 10 insecticides to one another are similar against both immature and adult stages of *H. coagulata.* However, these data do not reflect field efficacy, because these are based on direct dose–mortality relationships to a number of concentrations under laboratory conditions. But the information presented here is important in serving as a comparison point of relative toxicity to these insecticides.

This study provided data on the differential sensitivity of *H. coagulata* life stages to various insecticides that can be used in control programs to manage this pest. The higher activity of some compounds compared with others makes them an ideal option for *H. coagulata* control. Our results have shown that the period of greatest sensitivity to insecticides for *H. coagulata* is during the first two instars and that maximum efficiency from insecticide treatments should be exploited during these life stages. Conceivably, chemicals could be more effective if used against the early instars than at the adult stage. However, under field conditions, because both adults and larvae of *H. coagulata* are present and would normally receive the same treatment; any dose calculated to kill adults will also kill the immatures and thus will suppress the next generation. Higher dosages cause mortality at earlier stages of development. Appropriate timing of chemical application is essential for effective control of *H. coagulata* in the field. This information can be used to optimize present control measures that are in place to manage glassy-winged sharpshooter populations.

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